



## HEPATOTOXIC EFFECT OF TARTRAZINE AND ERYTHROSINE ON MALE WISTAR RATS

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### ABSTRACT

Recently, progressive use of synthetic food additives has paid more attention on their benefit and less attention on their toxic effect. Tartrazine and erythrosine are synthetic food colorants widely used in food, cosmetics and pharmaceuticals industries. This study is aimed at evaluating the possible hepatotoxic effect of these synthetic food colorants. A total of 25 adult male albino rats were divided into five groups with 5 rats per group. Group 1 is control group and given only water and feed. While group 2,3,4,5 were administered 5mg/kgb.wt, 10mg/kgb.wt, 20mg/kgb.wt, 40mg/kgb.wt of Tartrazine and Erythrosine respectively. At the end of the experimental period (21 days), blood samples were collected via ocular puncture and used to measure the serum activity of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP), on the animals. The result revealed that there was a high significant difference  $p < 0.05$  in ALT activity of group4 ( $8.98 \pm 2.49$ U/L) when compared with the control ( $3.21 \pm 0.09$ U/L). Group5 also showed higher ALT activity ( $7.66 \pm 0.37$ U/L) when compared with group2 ( $1.98 \pm 0.18$ U/L). Also, AST activity in group5 was significantly higher ( $68.53 \pm 2.23$ U/L) when compared with the control ( $3.11 \pm 0.18$ U/L). The increase was observed across group. Furthermore, the result showed a high serum ALP activity in group4 ( $26.01 \pm 2.04$ U/L) when compared with the control ( $16.00 \pm 1.09$ U/L) and other groups. The above result shows that the combination of Erythrosine and Tartrazine is capable of increasing the serum activity level of the liver enzymes (ALT, AST and ALP). This implies that high and constant ingestion of these synthetic food colorants could be hepatotoxic, hence liver damage.

**KEYWORDS:** Erythrosine, Tartrazine, AST, ALT and ALP.

### 1.0 INTRODUCTION

People are always exposed in so many ways to many toxic or harmful substance. The toxicant can affect any tissue or organ in a human body system. Some of these toxicants are mainly present in our environment such as air, water, industrial waste, they are equally present in some of the food we eat. Tartrazine (E number E102) a synthetic azo dye with lemon yellow color, is a commonly used food colorant for food products that we eat almost every day.<sup>[1]</sup> Among foods containing tartrazine are soft drinks and sport drinks, flavored chips, sauces, ice creams, jams, jellies and chewing gums.<sup>[2]</sup> Tartrazine is found in many non-food consumables such as soaps, cosmetics, shampoos, vitamins and certain prescription medications.<sup>[3]</sup> Moreover, it is used in many developing countries as a low cost alternative for saffron in cooking.<sup>[4]</sup> Tartrazine is typically labeled as a colour because it gives the food a colour appealing and attractive to consumer.

Another synthetic food colourant, found in food is known as Erythrosine. Erythrosine also known as Red No. 3 is an organoiodine compound, it is cherry-pink in

colour used mainly for food.<sup>[5]</sup> It is commonly used in sweets such as; candies and even more widely used in cake-decorating gels.

The Liver is an organ only found in vertebrates, that detoxifies various metabolites, synthesizes proteins and produces biochemical necessary for digestion.<sup>[6]</sup>

### 2.0 MATERIALS AND METHOD

**2.1 Sample Preparation:** Tartrazine (T0388-100G) and Erythrosine (1159360025) which is a synthetic dye was gotten from sigma company United State of America. 5mg, 10mg, 20mg and 40mg of tartrazine and erythrosine was weighed using an electronic weighing balance. Then it was dissolved with distilled water of 84ml in a ratio of 1:1, Which was administered to group 2, 3, 4 and 5 respectively.

### 2.2 Animal Handling

The animal types used for this research work are healthy male red eyed albino rats of the wistar strain with an average weight range of 128- 244g. A total number of 25 rats gotten from Federal University of Technology

Owerri (FUTO) were used. The rats were acclimatized for one week to have proper adaptation to their environment before administration.

**2.3 Animal Weight:** The rats were randomly grouped into five groups with each group containing 5 rats according to their body weight.

**2.4 Administration of Dissolved Tartrazine and Erythrosine:** Group 1 (control) were administered distilled water. Group 2,3,4,5 were administered 5mg/l, 10mg/l, 20mg/l and 40mg/l of tartrazine and erythrosine. Each rat was administered on a daily basis according to their body weight for 21 days.

### 2.5 Sample Collection

After, the end of the experiment period (21 days).The animals were sacrificed and the blood samples were collected through ocular puncture and spun.The blood samples were separated into serum using a centrifuge. The samples were centrifuged at 5000 rpm for 10mins and the serum was collected into sterile bottles and preserved in the refrigerator.

### 2.6 Analysis

The separated serum were used to carry out the following analysis

- Alanine aminotransferase
- Aspartate transferase
- Alkaline phosphatase

### 2.7. Method of Assay and Assay Procedure

#### 2.7.1 Alanine Aminotransferase Assay Principle (Sgpt)

##### Principle

L-Alanine + alpha-ketoglutarate  $\longrightarrow$  pyruvate + L- glutarate  
 Pyruvate + NADH + H<sup>+</sup>  $\longrightarrow$  L-Lactate + NAD<sup>+</sup>  
 LDH- Lactate dehydrogenase

##### Procedure

100µl of sample is added to the sample tube  
 1000µl of the working reagent is added to both the sample tube  
 Mix and incubate at 37°C for one minute  
 Mix, read the change in absorbance of the sample during three minutes at 405nm wavelength

##### Calculation

ALT activity (U/L) = Change in Absorbance  $\times$  1745

#### 2.7.2 Alkaline Phosphatase (ALP)

##### Principle:

Para-nitrophenyl phosphate + H<sub>2</sub>O  $\longrightarrow$  P-nitrophenol + inorganic phosphate  
 ALP- Alkaline phosphatase

##### Procedures

100µl of sample is added to the sample tube

1000µl of the working reagent is added to both the sample tube

Mix and incubate at 37°C for one minute

Mix, read the change in absorbance of the sample during three minutes at 426nm wavelength

##### Calculation

ALP activity (U/L) = Change in absorbance  $\times$  2750

#### 2.7.3 Aspartate Aminotransferase(SGOT)

##### Principle

L-Aspartate + a-ketoglutarate  $\longrightarrow$  Oxaloacetate + L-Glutamate

Oxaloacetate + NAHH + H<sup>+</sup>  $\longrightarrow$  L-Malate + NAD<sup>+</sup>

AST- Aspartate aminotransferase

MDH- Malate dehydrogenase

##### Procedures

100µl of sample is added to the sample tube

1000µl of the working reagent is added to both the sample tube

Mix and incubate at 37°C for one minute

Mix, read the change in absorbance of the sample during three minutes at 405nm wavelength

##### Calculation

ALT activity (U/L) = Change in Absorbance  $\times$  1745

### 3.0 RESULTS

#### 3.1 Effect of Tartrazine and Erythrosine on Alanine aminotransferase Levels in male Wistar Rat

As shown in figure 3.1 below, statistically  $p < 0.05$ , Group 4 showed higher activity of ALT ( $8.98 \pm 2.49$  U/L) when compared to the control ( $3.21 \pm 0.09$ U/L), group 5 also show a high activity ( $7.66 \pm 0.37$ U/L) when compared to that of group 2 ( $1.98 \pm 0.18$ U/L). Group 4 was administered 20mg/kg, group 5 was administered 40mg/kg and group 2 was administered 5mg/kg.

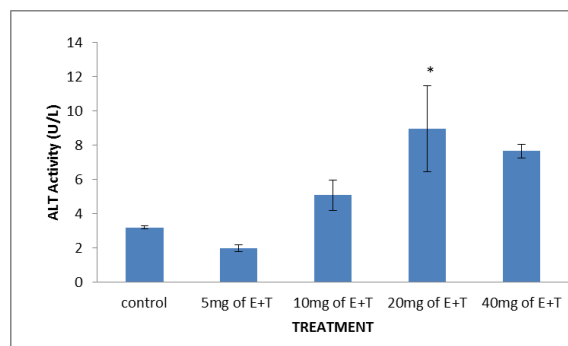
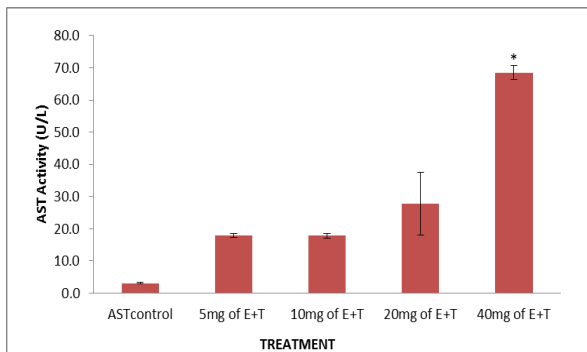


Figure. 3.1: Effect of Tartrazine and Erythrosine Administration on ALT levels on Wistar Rats.

#### 3.2 Effect of Tartrazine and Erythrosine on Aspartate aminotransferase activity on Male Wistar Rats.

As show in Fig 3.2 below, aspartate aminotransferase (AST) activities has significantly difference  $p < 0.05$  in group 5 ( $68.53 \pm 2.23$ U/L) when compared to that of the

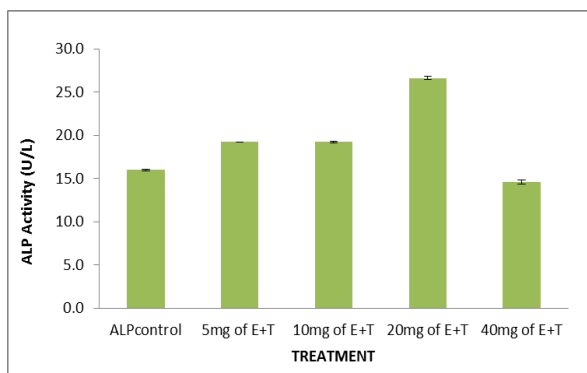
control ( $3.11 \pm 0.18$  U/L). this increased were observed in other groups.



**Figure. 3.2: Effect of Tartrazine and Erythrosine on Aspartate aminotransferase activity on Male Wistar Rats.**

### 3.3 Effect of Tartrazine and Erythrosine on Alkaline phosphatase activity on male wistar rats.

Fig 3.3 below shows that there was no significant difference  $p < 0.05$  in group 4 when compared to that of the control and other groups. However group 4 shows a higher ALP activity when compared to other group.



**Fig. 3.3: Effect of Tartrazine and Erythrosine on Alkaline phosphatase activity on Male Wistar Rats.**

### 4.1 DISCUSSION

Tartrazine and Erythrosine are azo dyes mainly used in the production of food and so many other product used around the world. These azo dyes helps in the enhancement of colour.<sup>[4]</sup> According to<sup>[3]</sup>, these synthetic food colourant have been seen to have effect on the hepatic cells of the liver.

These liver enzymes play an important role in the assessment of the liver function, because liver injury or damage resulting to liver cytolysis will cause the release of liver enzymes (ALP, ALT, AST) into the blood circulation, thus are biochemically known as markers of liver disease.<sup>[7]</sup>

From the result, the activities of hepatic serum enzymes (AST and ALT) increased in rats administered tartrazine and erythrosine particularly at high doses, suggesting elevated, injuries and impairment of the hepatic cells. Also, elevation in both ALT (located in the cytoplasm)

and AST (located mainly in organelles such as mitochondria) activities indicated the injury of both the hepatic cellular and mitochondrial membranes in food azo dyes administered rats.<sup>[8]</sup>

For Alanine aminotransferase (ALT) activities, the result revealed that there was a significant difference  $p < 0.05$  in ALT activity of group 4 ( $8.98 \pm 2.49$  U/L) when compared with the control ( $3.21 \pm 0.09$  U/L), group 4 which was administered 20mg/kg. Group 5 also showed a higher activity ( $7.66 \pm 0.37$  U/L) when compared to that of group 2 ( $1.98 \pm 0.18$  U/L), group 2 and group 5 were treated with 5mg/kg and 40mg/kg respectively.

For Aspartate aminotransferase (AST) activities, the result showed that the AST activity in group 5 was significantly higher ( $68.53 \pm 2.23$  U/L) when compared to that of the control ( $3.11 \pm 0.18$  U/L), the increased was observed in all the groups.

In this concern,<sup>[9]</sup> found that liver tissues which are known of their high contents of transaminases (AST, ALT) lose their enzymes in case of liver cells damage. The effect of these synthetic food colourant on the liver cells is in accordance with<sup>[10]</sup>,<sup>[11]</sup> and<sup>[12]</sup><sup>[13]</sup> who recorded a pronounced increase of serum and liver transaminases activity in rats ingested synthetic dyes. AST is considered to be more specific for heart function tests<sup>[14]</sup> which indicates that tartrazine has a retard damage effect on the heart function.

Increase in both serum AST and ALT of rats was attributed to the changes in liver function and hepatocellular impairment was subsequently caused the release of greater than normal levels of intracellular enzymes into the blood.<sup>[15]</sup>

Alkaline phosphatase (ALP) has several physiological functions in bone cells, it splits inorganic phosphates from organic phosphate which is a potent inhibitor of mineralization.<sup>[16]</sup> Furthermore, the result showed a high serum ALP activity in group 4 ( $26.01 \pm 2.04$  U/L) when compared to the control ( $16.00 \pm 1.09$  U/L) and was also observed in other groups. Alkaline phosphatase occurs in the canalicular and sinusoidal membranes of the liver, thus damage to the liver will result in elevated serum ALP activity.<sup>[3]</sup>

In addition,<sup>[17]</sup> reported that consumption of food colour including tartrazine and erythrosine induces hepatic tissue damages in Swiss Albino Rats.

The findings of<sup>[3]</sup> is in agreement with that of<sup>[18]</sup> who specified that high doses of synthetic dyes contains both erythrosine and tartrazine which revealed a significant elevation of serum ALT, AST and ALP activities these changes to hepatocellular injury produced by the toxic properties of these azo dyes that is associated with swelling, pyknosis, vacuolation and necrosis of the hepatic cells. The elevated activities of aminotransferases

with the histopathological changes suggested that the tissue impairment of mainly the liver, heart and kidney is associated with synthetic dyes. This implies that high and constant ingestion of these synthetic food colourant could be hepatotoxic, hence liver damage.

#### 4.2 CONCLUSION

The Liver is a vital and delicate organ of the body which perform numerous functions that are key for the normal body function. As such damage to the liver will lead to a lot of malfunctioning. The result review that, the two dyes in study increased the liver enzyme activities which is an indication that the dye is a potential risk factor for the liver damage. Precautions should be taken while taking dyes in food or liver sustaining agent (drug) may be required.

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